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Growth and Survival Evaluation of *Oreochromis* Sp fed *Hermetia illucens* Larva and *Manihot esculenta* leaves Meal

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Abstract

This study was conducted to compare the effects of dietary substitution of fish meal (FM) with black soldier fly (*Hermetia illucens*) larvae meal (BSFM) and *Manihot esculenta* leaves meal (MEM) on the growth and feed efficiency of *Oreochromis* sp. Four concentrations *viz*: P1 (25%): 50g BSFM and 25g MEM, P2 (50%): 100g BSFM and 50g MEM, P3 (75%): 150g BSFM and 75g MEM, P4 (100%): 200g BSFM and 100g MEM were prepared and tested against control without FM replacement. Each diet was fed to three replicates groups of fish at a rate of 5% of body weight two times per day for 30 days. At the end of the trial, growth parameters, Feed conversion rate (FCR), and feed efficiency (FE) were evaluated. The results showed that fish fed dietary substitution of FM with combination ratio of BSFM and MEM higher than 50% significantly improved all growth parameters, FCR and FE. It is therefore suggested that the partial (higher than 50%) or total replacement of fish meal with combination of BSFM and MEM in the diet of *Oreochromis* sp can be used as fish meal substitution to obtain better growth and feed efficiency.

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INTRODUCTION

Recently, the global production of tilapia has reached 5.000.000 metric tons, placing this sector in the second largest aquaculture in the world (Fitzsimmons, 2016). As one of the pivotal species in Indonesia aquaculture, the production of tilapia has also enhanced gradually in some Indonesia's region such as South Sumatera, West Java and East Kalimantan (Arisanti *et al.*, 2013). In addition, the enhancing market demand of tilapia has triggered research about nutrition in order to boost productivity and health performance.

Nutrition in aquaculture is a high cost component, particularly in intensive culture. Reaching over 50% of operating budget. Protein as one of the important nutrients provides amino acids which is required for building new tissue and/or replacing old tissues. The protein component alone in fish diets represents about 50% of feed cost in intensive culture (Kordi, 2010). The research conducting the use of protein from insect as a promising foodstuff for animal has been done for almost 40 years ago. However, the supplementation of protein from insect as aquafeed has not been widely explored by researchers. The protein from insect can be considered as a substitution of fish meal (FM) because of environmentally friendly and has high protein and lipid (Hanief *et al.*, 2014; Khosravi *et al.*, 2015; Sumiati *et al.*, 2006; Tang *et al.*, 2009). Past research on the substitution of protein from insect in aquafeed is very limited. Nevertheless, over the last few years, the interest of protein insect as aquafeed has enhanced, and many feeding trials have been done at many kinds of fish both in carnivorous and herbivorous or even omnivorous fish. Previous studies revealed that the supplementation of low cost reference diets for fish has been done using protein from whole or parts of insect which is transformed into an insect protein meal (AOAC, 1990; Effendi *et al.*, 2006; Iskandar, 2015), for example yellow mealworm (*Tenebrio molitor*) (Setiawati, 2008). Tough the adult's stage of insect may not consider to be applied in feeds as they contain quinones; the larvae stage of insect has a high nutritional value, containing high protein and lipids, but low in ash (Craig & Helfrich, 2002).

Previous study on the larvae of *T. molitor* (TM) revealed that the larvae can be potentially used as a meal (Craig & Helfrich, 2002; Setiawati, 2008) and recently being produced at a huge industrial scale in China (Boyd, 1998). The TM can be able to be used either partial replacement in fish feed by sun-dried or full replacement. Some past research regarding insect meal substitution have been also done in some fish such as African catfish (*Clarias gariepinus*) (SNI, 2015); *Ameiurus melas* (Roncarati *et al.*, 2014a; Roncarati *et al.*, 2014b), rainbow trout (*Oncorhynchus mykiss*) (Gasco *et al.*, 2014). The substitution FM with larvae *T. molitor* at level 20% found to be a positive effect on the growth of African catfish and resulted a significant enhancement in comparing with fish without substitution (Gasco *et al.*, 2014). Another study, however, revealed that substitution FM with TM at a high levels between 80 and 100% resulted to decrease fish growth performance, feed and protein efficiency (Ng *et al.*, 2001). In addition, the use of TM meal to replace of 100% of FM significantly reduced fish growth performances in comparison with 50% replacement of FM (Roncarati *et al.*, 2014a; Roncarati *et al.*, 2014b).

Another insect that potentially can be use as FM substitution is larvae of Black Soldier (*Hermetia illucens*). The Black soldier fly larvae in the form of meal (BSFM) is also potentially used to replace FM in diets for some fish such as European seabass (*Dicentrarchus labrax*) (Magalhães *et al.*, 2017); Yellow catfish (*Pelteobagrus fulvidraco*) (Hu *et al.*, 2017); and juvenile Jian carp (*Cyprinus carpio* var. Jian) (Li *et al.*, 2017). The BSFM is valuable ingredient, low cost, and has similar protein value, approximately 36-48% (Hu *et al.*, 2017).

Besides protein source from larva, leaves of cassava (*Manihot esculenta*) can be also made as meal (MEM) to substitute FM. Leaves of cassava as high protein from 22,6 to 30%(Syahrizal *et al.*, 2017). Past study stated that the growth of *Clarias gariepinus* was optimum after fed 25% inclusion in the fish diet (Odo *et al.*, 2016). In contrary, Aride *et al.* (2016) found that diet for Tambaqui (*Colossoma macropomum*) consisting of 30% cassava reduced weight gain and specific growth rate.

Growth statues are a major parameter tool in aquatic management to evaluate growth performance of cultured fish. The ingredient in the aquafeed is one of the most important factors influencing the ability of cultured fish to exhibit its genetic potential for growth (Ali *et al.*, 2015). Growth statues can be evaluated by calculating weight gain, body weight gain (BWG), daily weight gain (DWG), average weekly gain (AWG), specific growth rate (SGR), thermal growth coefficient (TGC), and Length gain which are the simplest parameters to determine the growth statues of fish (Busacher *et al.*, 1990; Nur *et al.*, 2017; Yusup & Nugroho, 2017). Besides growth parameters, feed efficiency (FC) and food conversion ratio (FCR) are also important tools to determine the effectiveness of the diets. (Ali *et al.*, 2015). The FCR is a measure of an animal's efficiency in converting feed mass into body mass.

Although the research regarding the effects of FM replacement with protein from insect larva and MEM in the diet on some fish has been done, the role of the effects of BSFM in combination with MEM on the growth statues and feed efficiency of tilapia (*Oreochromis* sp) is limited know. Thus, the aim of the current research was to evaluate the growth statues including BWG, DWG, AWG, SGR, TGC, Length gain, and feed efficiency of tilapia after fed FM replacement with TM and MEM.

METHODS

Black Soldier Fly and *Manihot esculenta* meal preparation

Collected BSF larva were sacrificed by freezing (Finke *et al.*, 1989) and immediately dried in an oven at 60°C for 20 h. The BSF larva were powdered using manual grinding machine. The resulting powder was packed and stored in a dark room before being used as a test diet. To prepare *Manihot esculenta* meal (MEM), leaves of cassava were washed and soaked for 24h to eliminate HCN. The leaves were then cut into small pieces and dried using oven at 60°C until dried. The dried leaves were then grinded to make a powder. The MEM was kept in the dark plastic and stored at 4°C until used as test diet.

Diet preparation

A control and tests diet were prepared to satisfy the nutrient requirements of *Oreochromis* sp. Five test diets were formulated as described in Table1. The test ingredients were partially replacement of FM with combination ratio of BSFM and MEM at levels 25(P1), 50(P2), 75(P3), and 100%(P4). The control diet (0%): was a diet without BSFM and MEM, P1 (25%): 50g BSFM and 25g MEM, P2 (50%): 100g BSFM and 50g MEM, P3 (75%): 150g BSFM and 75g MEM, P4 (100%): 200g BSFM and 100g MEM All test feed ingredients were obtained from commercial sources in Indonesia.

Diet proximate composition

Proximate composition of the diet was conducted according to the standard methods described by the Association of Official Ana-

Table 1. Composition of test diets used for experiment in tilapia, *Oreochromis* sp

Ingredients (%)	Groups				
	Control	P1	P2	P3	P4
Fish meal	30	22.5	15	7.5	0
Shrimp meal	20	20	20	20	20
Corn starch	10	10	10	10	10
Wheat grain	22.5	22.5	22.5	22.5	22.5
Tapioca flour	10	10	10	10	10
BSFM	0	5	10	15	20
MEM	0	2.5	5	7.5	10
Vitamin premix	0.5	0.5	0.5	0.5	0.5
Mineral premix	3	3	3	3	3
Corn oil	4	4	4	4	4
Moisture	1.65	1.59	1.68	1.09	1.27
Ash	9.2	9.24	9.13	9.71	10.23
Fat	15.33	17.05	16.7	17.44	17.54
Protein	30.21	26.59	23.58	24.49	29.56
Fiber	11.29	12.42	12.94	16.33	16.25
Carbohydrate	25.78	33.12	35.98	30.95	25.15

Note:All dry diet components, including vitamins and minerals mixture was thoroughly mixed with corn oil. Water was added, and the feed pressed into pellets of 1 mm diameter. The wet pellets were dried for 3 days at room temperature and stored at 4°C until use. Control (0%): without Black Soldier Fly Larva Meal (BSFM) and Cassava Leaves Meal (MEM), P1 (25%): 50g BSFM and 25g MEM, P2 (50%): 100g BSFM and 50g MEM, P3 (75%): 150g BSFM and 75g MEM, P4 (100%): 200g BSFM and 100g MEM lytical Chemists: Moisture was determined by drying the samples in an oven (Behr, Germany) at 105 °C; Crude lipid was determined by chloroform methanol extraction (2:1, v/v); Crude protein was determined (Kjeldahl procedure: N x 6.25) using an automatic Kjeldahl system; Ash was measured by incineration in a muffle furnace at 500 °C for 6 h.

Animal and Experimental Setup

A total of 150 healthy tilapia (average initial weight ± 5 g; initial length ±7 cm) was stocked in the current experiment. All fish were acclimated in the laboratory for at least seven days. During the time of acclimatization fish fed on a commercial pellet diet twice per day (09:00, 17:00 GMT) at a rate of 5% body weight per day. The compositions of test diet are provided in Table 1. After acclimatization, fish were randomly divided into five groups. Each group consisted of three replicates of 10 fish per tank. The first group was fed on commercial-test diet (Control) and the remaining group were fed on test diets (P1-P4, ranging the replacement of FM from 25, 50, 75, to 100%) (Table1). The experiment was lasted for 30 days. Dissolved oxygen (mg L^{-1}) (Lutron DO-5509) and pH were monitored using a CyberScan pH 300 (Eutech Instruments, Singapore, China), temperature (°C) were monitored and measured by using routine thermometer every two days in each tank during the experimental period. Experimental tanks were regularly cleaned, and the fecal matters was siphoned out daily.

Growth Performance measurement

The weight of all fish from each tank was measured at initial and final day of the trial. After the feeding trial, the growth parameters including, final weight, body weight gain (BWG), daily weight gain (DWG), average weekly gain (AWG), specific growth rate (SGR), final length, length gain, daily length gain (DLG), Fulton condition and thermal growth coefficient (TGC). The TGC was calculated by using equation as follow:

$$TGC = \frac{[\sqrt[3]{W_t} - \sqrt[3]{W_0}]}{[\text{water temperature x day of culture}]} \text{ x } 100$$

where: W_t = weight of fish at the end of trial (g); W_0 = Initial weight (g)

To estimate feed efficiency, the parameter of feed conversion rate (FCR), and feed efficiency (FE) were also calculated. The BWG (g per fish), DWG, AWG (g w^{-1}), SGR (% g day^{-1}), and survival are calculated using equation as previously used by Wang (2007); (Yusup & Nugroho, 2017); and Sang *et al.* (2011). The weight of each fish from each tank was measured using electronic balance (GX-4000, A&D Company, Ltd., Japan).AWG = [final weight (g) - initial weight (g)]/week. SGR = 100 x ([Ln(Wt) – Ln(Wo)]/d) where Wt and Wo are the weight of the fish at current time (t) and at the commencement of the experiment (0), d = Culture period (day), respectively. FCR= F/Wg, where FCR= feed conversion ratio, F= total of feed intake(g), Wg= weight gain during the study (g).The feed efficiency (FE)= (1/FCR) x 100 (Muchlisin *et al.*, 2016).

Statistical Analysis

All data was statistically performed by using SPSS for Windows (Version 22) and expressed as mean ± SE (standard error). Percent data of survival was normalized using an arcsine transformation before performing significant differences analysis. The data was subjected to one-way analysis of variance (ANOVA) to find any significant differences among various tested parameters. To determine the significant differences, Duncan's multiple range *post hoc* tests at a significance level of $P<0.05$ was used.

RESULTS & DISCUSSION

The growth parameters of fish fed the different substitution levels of FM with BSFM and MEM in the diets are shown in Table 2. Current findings stated that all growth parameters such as final weight , BWG, DWG, AWG, SGR, and TGC of fish fed diet with FM replacement higher than 50% were similar to fish control group without FM substitution. The BWG of fish fed total replacement FM in combination between 200 g BSFM and 100 g MEM showed the highest BWG (11.784±0.386). The final length, length gain, and Fulton condition of fish fed with FM substitution in combination of BSFM and MEM up to 100% also found better than fish without FM replacement. This result indicated that FM in the diet of *Oreochromis* sp can be partially or totally replaced by combination of BSFM and MEM.

Besides growth parameters, feed efficiency of fish fed FM replacement with BSFM and MEM higher than 50% also showed better FCR and FE (Table 3). The best FCR was obtained in the group of fish fed with total replacement of FM, containing 200g BSFM and 100g MEM. The FE also reached 56,89%, showing better than control group which only 43,95%. These findings also indicated that total substitution of FM with 200g BSFM and 100g MEM can be alternatively used to replaced FM in the diet of *Oreochromis* sp.

Table 2. Growth parameter of *Oreochromis* sp. fed dietary *Hermetia illucens* L. larvae meal and *Manihot esculenta* leaves meal as fish meal substitution for 30 days

Parameter	Groups				
	Control 0%	P1 (25%)	P2 (50%)	P3 (75%)	P4 (100%)
Initial weight (g)	4.722±0.087	3.140±0.095	3.223±0.093	3.761±0.155	3.862±0.171
Final weight (g)	15.095±0.529^{a}	12.181±0.389^{b}	12.023±0.468^{bc}	14.943±0.594^{acd}	15.647±0.509^{acd}
BWG (g/ fish)	10.373±0.480^{a}	9.041±0.313^{b}	8.800±0.388^{bc}	11.182±0.456^{acd}	11.784±0.386^{acd}
DWG (g/ fish)	0.371±0.017^{a}	0.323±0.011^{b}	0.314±0.014^{bc}	0.399±0.016^{acd}	0.421±0.014^{acd}
AWG (g/fish)	2.593±0.120^{a}	2.260±0.078^{b}	2.200±0.097^{bc}	2.796±0.114^{acd}	2.946±0.097^{acd}
SGR (%)	4.100±0.109^{a}	4.841±0.057^{b}	4.671±0.065^{bc}	4.932±0.060^{acd}	5.035±0.090^{abcd}
TGC	0.441±0.020^{a}	0.385±0.013^{b}	0.374±0.016^{bc}	0.477±0.019^{acd}	0.502±0.016^{abcd}
Initial length	6.992±0.076^{a}	6.323±0.088^{b}	6.114±0.079^{bc}	6.445±0.114^{bcd}	6.567±0.097^{bce}
Final length	9.589±0.128^{a}	8.797±0.143^{b}	8.855±0.132^{bc}	9.354±0.170^{acd}	9.655±0.141^{abcd}
Length gain	2.597±0.094^{a}	2.474±0.114^{b}	2.740±0.079^{ab}	2.908±0,086^{cd}	3.090±0.084^{cde}
DLG	0.087±0.003^{a}	0.082±0.004^{b}	0.091±0.003^{ab}	0.097±0.003^{cd}	0.103±0.003^{cd}
K	1.702±0.038^{a}	1.805±0.054^{b}	1.714±0.021^{ab}	1.822±0.036^{bcd}	1.736±0.033^{abc}

Note: Results were expressed as mean ± standard error. Different superscript alphabets in the same row indicate significantly different means *P<0.05* BWG = Body weight gain; DWG = Daily weight gain; AWG = Average weekly gain; SGR = Specific growth rate; TGC = Thermal growth coefficient, DLG = Daily length gain; K = Fulton condition. Control (0%): without black Soldier Larva Meal (BSFM) and Cassava Leaves Meal (MEM), P1 (25%): 50 g BSFM and 25 g MEM, P2 (50%): 100 g BSFM and 50 g MEM, P3 (75%): 150 g BSFM and 75 g MEM, P4 (100%): 200 g BSFM and 100 g MEM.

Table 3. Feed conversion ratio and feed efficiency of *Oreochromis* sp. fed different combination ratio of *Hermetia illucens* L. larvae and *Manihot esculenta* leaves meal for 30 days

Parameters	Groups				
	Control 0%	P1 (25%)	P2 (50%)	P3 (75%)	P4 (100%)
FCR	2.44±0.15^{a}	1.96±0.05^{b}	2.08±0.082^{ab}	1.93±0.06^{bc}	1.85±0.08^{bcd}
FE(%)	43.95±2.07^{a}	52.23±1.83^{b}	50.08±1.93^{ab}	53.36±2.07^{bc}	56.89±2.66^{bcd}

Note: Results were expressed as mean ± standard error. Different superscript alphabets in the same row indicate significantly different means *P<0.05*.FCR = Feed Conversion Ratioand FE = Feed Efficiency. Control (0%): without Black Soldier Fly Larva Meal (BSFM) and Cassava Leaves Meal (MEM), P1 (25%): 50g BSFM and 25g MEM, P2 (50%): 100g BSFM and 50g MEM, P3 (75%): 150g BSFM and 75g MEM, P4 (100%): 200g BSFM and 100g MEM.

One of the pivotal factors that need to be done in the aquafeed to enhance aquaculture extension is the acquisition and feed price (Moradi *et al.*, 2013). Numerous scientist recommended different protein sources for the preparation of aquafeed to obtain maximum growth of fish (Kaushik & Troell, 2010; Metian, 2009; Radhakrishnan *et al.*, 2016). Protein as a major ingredient in fish feed derives from FM which is highly digestible (up to 90%) and without or less anti-nutritional factors (ANFs) (Gatlin III *et al.*, 2007). Nevertheless, the use of FM in the diet has increased cost of feed, not environmentally friendly, and competed with human need. Thus, the efforts to replace FM with other source protein either from animal or plant have been done and successfully conducted (Nugroho & Nur, 2018).

Present results stated that combination of BSFM and MEM to replace up to 100% of FM increased growth performance and feed efficiency. This result is similar to previous study revealed that replacement FM with Maggot meal-based diets compared favorably with fish meal-based diets and found to be no significant differences in the growth performance in African catfish (*Clarias gariepinus*) (Aniebo *et al.*, 2018). Another study also found that the replacement of FM with prepupae BSFM in diets at level of 19.5% of HM to replace 45% of FM did not affect the growth performance of European seabass (*Dicentrarchus labrax*) (Magalhães *et al.*, 2017).

Barroso *et al.* (2014) stated that most of the insect's species have a high nutrition value of protein which is similar to the levels of soy meal but lower than fish meal. For example, Diptera has the levels of crude protein range from 40 to 50%, especially *Hermetia illucens* has protein around 36.2 – 37.8 % (Arango Gutiérrez *et al.*, 2004; Sheppard, 2002). Though insect-based protein has several advantages, there is a problematic on the use of protein from insect that contains nitrogen within nacetylglucosamine, a subunit of the chitin polymer, difficult to digest digestibly (Finke *et al.*, 1989). Further, past study also found that the protein content in the insects is generally lower than fish meal but similar to soybean meal. In addition, some insects are also lacks certain amino acid (AA). Insects, *Zonocerus variegatus* and *Macrotermes bellicosus,* are deficiencies in certain AA such as Methionine, Lysine, threonine or tryptophan. Thus, it is suggested that to replace FM should be in combination with other source of protein that may help to prevent the deficiencies caused by the use of a single ingredient and to enhance growth performance (Hansen *et al.*, 2007; Hansen *et al.*, 2011; Zhang *et al.*, 2012a; Zhang *et al.*, 2012b).

Current findings also found that *Oreochromis* sp fed with BSFM in combination with MEM had significantly better growth performance and feed efficiency. This result is similar to past research revealed that replacing fish meal by a mixture of different plant protein sources showed better growth performance in Nile Tilapia (*Oreochromis niloticus* L.) (Al-Thobaiti *et al.*, 2018). Some studies also revealed that the used of soybean meal (Mo *et al.*, 2016), cotton seed meal (Yu *et al.*, 2014), seaweed (*Gracillaria arcuate*) (Al-Asgah *et al.*, 2016) have significant effects on the growth of fish. The FCR and FE both are related to dietary protein intake and can be converted into fish weight gain (Koumi *et al.*, 2009). Present experiment also revealed that there were significant effects on the FCR and FE among the four experimental diets and the commercial diet (Control) which were fed to *Oreochromis* sp. In line with this finding, Radhakrishnan *et al.* (2016) stated that in the total replacement of FM by *Chlorella vulgaris* had significantly improved the growth and energy utilization of *Macrobrachium rosenbergii*. In addition, the FCR enhanced with an increase in fish weight (Al Hafedh, 1999; Siddiquiaq & Adam, 1988). In addition, De Silva and Anderson (1995) the FCR for fish fed well prepared diets ranges between 0.46 and 1.15. Ogunji and Wirth (2000) revealed that FCR 1.19 indicated the most efficient utilization of feed by Nile tilapia. Other research revealed that FCR highest than 1.5 has been observed (Chou *et al.*, 2001)

In contrast, some research stated that plant-based protein still has antinutritional factors that potentially reduced growth performances and other negatives effects on the fish.Torstensen *et al.* (2008) found that Specific growth rate of Atlantic salmon (*Salmo salar*) was significantly lower in the combined high replacement of FM with plant meal. This reduce of the SGR might be caused by the occurrence of antinutritional (Kumar *et al.*, 2012). Antinutritional from plant can be defined as substances that can interfere with food utilization and affect the health and production in animals (Makkar, 1993).

In consideration with the existence of antinutritional in plant, combination between BSFM and MEM to replace FM is the alternative way to obtain optimum fish growth and feed efficiency. Combination between insect-based protein and plant-based protein is also useful to balance the AA composition (Henry *et al.*, 2015). Nutritional studies have stated that combining superworm meal with 10% of a prebiotic mushroom in a fish diet further enhanced the performances of Growth Rate and FCR of fish (Din *et al.*, 2012) which also due to a balancing of the AA composition of the fish diet (Kim *et al.*, 2009).

CONCLUSION

The replacement of fish meal up to 100% in combination between black soldier fly meal and Manihot esculenta meal is suggested to get better growth performance and feed efficiency in Oreochromis sp. Further research needs to be conducted to determine the effects of replacement for long period to growth performance, immune profiles, and antioxidant enzymes activity.

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